

Table S1. The number of peaks and estimated FDR.

	Lim1 ($\lambda=1.72$)		Otx2 ($\lambda=1.95$)		gsc ($\lambda=0.82$)		p300 ($\lambda=1.12$)		TLE ($\lambda=1.14$)	
fold (IP / WCE) =	total peak	estimated FDR	total peak	estimated FDR	total peak	estimated FDR	total peak	estimated FDR	total peak	estimated FDR
1	1,424,080	0.5129401	1,160,072	0.5802915	1,490,549	0.1984144	1,008,378	0.3082868	432,907	0.3155873
2	406,546	0.2480654	356,116	0.3097929	467,705	0.05034127	357,326	0.1036441	176,742	0.1077689
3	108,991	0.09620397	155,203	0.1339688	154,530	0.009867946	173,184	0.02724421	114,231	0.0287979
4	33,598	0.03090354	93,599	0.0482546	45,105	0.001570916	104,896	0.00585223	86,374	0.006291168
5	11,245	0.008440191	67,194	0.01482604	25,002	0.000210203	73,783	0.001060425	69,897	0.001159633
6	4,946	0.002000698	52,768	0.003961765	8,657	2.42E-05	56,270	0.000165955	59,430	0.000184641
7	2,911	0.000418423	43,140	0.000935287	3,742	2.45E-06	45,434	2.28E-05	50,887	2.59E-05
8	1,849	7.82E-05	36,236	0.000197583	2,186	2.21E-07	37,792	2.80E-06	45,175	3.23E-06
9	1,362	1.32E-05	31,138	3.77E-05	1,445	1.80E-08	31,482	3.11E-07	40,303	3.64E-07
10	958	2.04E-06	27,095	6.58E-06	1,162	1.33E-09	26,939	3.13E-08	36,403	3.74E-08

λ = the expected tag number in the 120-bp window calculated by the WCE sample.
 colored cells, used in this study.

Table S2. Comparisons between our peaks and MACS peaks.

	our peaks									MACS peaks						
	total peaks			potential CRMs			CRMs with Otx2 and TLE			total		overlap**		no overlap		P-value‡
	number	overlap*	ratio	number	overlap*	ratio	number	overlap*	ratio	number	FDR†	number	FDR†	number	FDR†	
Otx2	27,095	26,628	98.3%	18,363	18,188	99.0%	14,075	13,956	99.2%	47,258	0.70%	21,870	0.15%	25,388	4.77%	<2.23E-308
Lim1	11,245	5,420	48.2%	5,562	3,943	70.9%	3,219	2,418	75.1%	8,351	11.93%	4,614	5.73%	3,737	17.14%	<2.24E-245
Gsc	25,002	10,757	43.0%	4,383	3,984	90.9%	3,386	3,195	94.4%	32,934	9.13%	10,491	5.74%	22,443	10.35%	<2.23E-308
p300	26,939	25,492	94.6%	13,882	13,144	94.7%	7,144	6,750	94.5%	44,945	0.22%	19,670	0.00%	25,275	0.75%	<2.23E-308
TLE	36,403	35,728	98.1%	17,470	17,293	99.0%	13,538	13,444	99.3%	61,674	0.86%	28,141	0.02%	33,533	10.26%	<2.23E-308

Note: Our peaks do not overlap with about a half of MACS peaks. These MACS peaks have much more false discovery rates (FDR) than those overlapped with our peaks, indicating that many of the relatively less reliable MACS peaks were excluded from our peaks.

*, overlap with MACS peaks

** , overlap with our peaks

† , median of FDR for each peak

‡ , chi-square test for FDR between overlap and no overlap peaks

Table S3. RNA-seq results for identification of head organizer genes.

Xtevm_id	length	rpkm (head organizer)	rpkm (remaining regions)	head organizer /remaining regions	gene name / synonyms	gene type (structure / family)	expression area in early gastrula
Xtevm_15.41.1_VX	1870	1120.69	79.03	14.2	frzb	secreted/sfrp	organizer
Xtevm_185.10.1_VV	1109	922.38	64.12	14.4	gooseoid	transcription factor/homeobox	organizer
Xtevm_68.12.1_VVA	2284	674.21	64.48	10.5	otx2	transcription factor/homeobox	organizer
fixed gene model 1	1285	545.01	19.42	28.1	cerberus	secreted/dan	organizer
Xtevm_444.2.1_VV	2110	388.71	24.46	15.9	hhex	transcription factor/homeobox	organizer
Xtevm_150.21.1_VV	752	362.75	8.11	44.7	dkk1	secreted/dickkopf	organizer
Xtevm_481.14.1_VXA	2728	343.24	66.41	5.2	otx5/crx	transcription factor/homeobox	organizer
Xtevm_112.13.1_VV	2203	327.87	20.35	16.1	admp	secreted/TGFb	organizer
Xtevm_133.1.1_VV	3122	304.19	19.53	15.6	frizzled8	transmembrane/receptor	organizer
Xtevm_44.9.1_VX	899	281.43	3.48	80.9	crescent	secreted/sfrp	organizer
Xtevm_63.14.1_VV	1544	256.13	40.29	6.4	gata4	transcription factor/zinc finger	dorsal endoderm
Xtevm_84.14.1_VV	4239	251.75	44.67	5.6	gata6	transcription factor/zinc finger	dorsal endoderm
Xtevm_132.27.1_VV	3079	227.72	23.27	9.8	chordin	secreted	organizer
Xtevm_873.5.1_VV	1832	226.19	39.92	5.7	cndp2/darmin-r	dipeptidase	unknown
Xtevm_25.18.1_VV	2630	205.49	6.61	31.1	pkdccc.2	kinase	unknown
Xtevm_269.6.1_VV	4305	177.75	33.96	5.2	prdm1/blimp1	transcription factor/zinc finger	organizer
Xtevm_17.11.1_VV	1568	167.86	24.37	6.9	LOC100494211	secreted	unknown
Xtevm_2460.2.1_VX	488	155.1	10.67	14.5	mig30	secreted/IGFBP	organizer
Xtevm_42.16.1_VV	4574	140.66	26.36	5.3	ephrlinb2	transmembrane/ligand	unknown
Xtevm_686.7.1_VX	628	139.8	3.43	40.8	dkkx	secreted/dickkopf	unknown
Xtevm_516.6.1_VV	2769	136.48	22.39	6.1	lim1/hx1	transcription factor/homeobox	organizer
Xtevm_95.11.1_VV	1023	134.88	3.09	43.7	rihA	ribonucleoside hydrolase	unknown
Xtevm_159.17.1_VV	3916	114.75	18.87	6.1	slc38a3	amino acid transporter	unknown
Xtevm_119.23.1_VV	1615	112.82	14.82	7.6	mmp1	secreted/metallopeptidase	unknown
Xtevm_317.7.1_VV	1308	90.37	5.73	15.8	noggin	secreted	organizer
Xtevm_289.4.1_VV	1534	82.96	5.29	15.7	cf/ami	secreted/complement factor	unknown
Xtevm_123.16.1_VX	712	78.84	11.78	6.7	tmem150b	transmembrane	unknown
Xtevm_281.18.1_VV	1040	63.62	11.24	5.7	pr5f	proline rich protein	unknown
Xtevm_110.12.1_VV	2511	52.5	6.78	7.7	tolloid-like1	secreted/metallopeptidase	unknown
fixed gene model 2	1474	50.59	2.84	17.8	siamois	transcription factor/homeobox	organizer
Xtevm_973.5.1_VU	1596	49.39	6.74	7.3	slc13a3	dicarboxylate transporter	unknown
Xtevm_89.17.1_VVA	2310	47.7	7.39	6.5	pitx2	transcription factor/homeobox	organizer
Xtevm_117.23.1_VX	1394	47.36	0.99	47.8	gjb1	transmembrane/connexin	unknown
Xtevm_898.1.1_VX	144	42.98	0.27	159.2	unknown	unknown	unknown
Xtevm_8.17.1_VV	317	42.55	7.35	5.8	cnrp1	cannabinoid receptor interacting protein	unknown
Xtevm_1132.1.1_VX	1696	37.41	2.49	15.0	ets1	transcription factor/ets	unknown
Xtevm_316.4.1_VV	1688	35.91	4.97	7.2	nkx6-2	transcription factor/homeobox	unknown
Xtevm_154.4.1_VX	1922	35.41	1.4	25.3	slc6a8	transmembrane/creatine transporter	unknown
Xtevm_34.38.1_VX	1275	33.52	4.25	7.9	nodal/Xnr3	secreted/TGFb	organizer
Xtevm_512.18.1_VV	2054	33.04	4.45	7.4	hnf1b	transcription factor/homeobox	dorsal endoderm
fixed gene model 3	3517	30.11	3.78	8.0	neuropilin2	transmembrane/receptor	unknown
Xtevm_165.18.1_VV	3613	28.3	4.4	6.4	vcan	ECM/chondroitin sulfate proteoglycan	unknown
Xtevm_145.1.1_VV	2492	28.24	4.45	6.3	foxa1	transcription factor/forkhead	dorsal endoderm
Xtevm_202.15.1_VV	1523	28.09	5.5	5.1	tiki1/c2orf89-like	transmembrane/protease	organizer
Xtevm_271.7.1_VV	985	26.26	2.14	12.3	nkx2-3	transcription factor/homeobox	unknown
Xtevm_22.25.1_VX	3144	22.01	1.21	18.2	anpep	transmembrane/aminopeptidase	unknown
Xtevm_191.9.1_VXA	777	21.99	1.57	14.0	prdm12	transcription factor/zinc finger	unknown
Xtevm_17.12.1_VV	3509	20.17	3.75	5.4	hps4	unknown	unknown
Xtevm_700.6.1_VV	1113	19.72	2.35	8.4	follistatin	secreted	organizer
Xtevm_1.13.1_VX	1085	19.45	0.66	29.5	dmbx1	transcription factor/homeobox	unknown
Xtevm_450.2.1_VX	1062	18.7	0.91	20.5	hao1	hydroxyacid oxidase	unknown
Xtevm_89.18.1_VV	477	18.4	3.27	5.6	unknown	unknown	unknown
Xtevm_329.17.1_VV	2642	18.22	1.12	16.3	gjb2	transmembrane/connexin	dorsal endoderm
Xtevm_83.10.1_VV	934	18.1	0.85	21.3	unknown	unknown	unknown
Xtevm_27.1.1_VV	2384	16.84	2.94	5.7	ras-dva	GTPase/Ras	unknown
Xtevm_197.12.1_VV	1417	14.67	2.78	5.3	LOC100158459	unnamed	unknown
Xtevm_154.19.1_VV	2577	13.98	2.49	5.6	st6galnac4	sialyltransferases	unknown
Xtevm_180.4.1_VV	1427	13.95	2.22	6.3	LOC100145040	unknown	unknown
Xtevm_486.1.1_VX	814	13.9	0.25	55.6	nkx2-2	transcription factor/homeobox	unknown
Xtevm_68.42.1_VV	1412	13.64	1.46	9.3	six1	transcription factor/homeobox	unknown
Xtevm_68.40.1_VX	1258	13.55	1.34	10.1	six6	transcription factor/homeobox	unknown
Xtevm_188.3.1_VV	957	13.27	2.34	5.7	zchc14	transcription factor/zinc finger	unknown
Xtevm_614.6.1_VV	3289	12.83	1.44	8.9	merlk	kinase	unknown
Xtevm_59.12.1_VU	1122	12.76	1.84	6.9	phox2b	transcription factor/homeobox	unknown
fixed gene model4	654	12.49	1.36	9.2	siamois2/twin	transcription factor/homeobox	organizer
Xtevm_23.24.1_VV	2865	10.45	1.86	5.6	spns2	transmembrane/sugar transporter	unknown
Xtevm_375.1.1_VV	902	10.03	0.08	125.4	rippy2	rippy	unknown
Xtevm_346.7.1_VV	1222	9.48	1.45	6.5	cry2l	cryptochrome	unknown
Xtevm_280.18.1_VV	1786	8.92	0.92	9.7	fgfr1/houdarake	transmembrane/FGF receptor like	organizer

Table S3 (continued)

Xtevm_66.41.1_VV	3209	8.78	1.09	8.1 cadherin11	transmembrane	unknown
Xtevm_68.14.1_VV	492	8.17	1.19	6.9 unknown	unknown	unknown
Xtevm_319.8.1_VU	3610	7.83	0.25	31.3 atp13a4l	atpase	unknown
Xtevm_289.7.1_VV	1008	7.6	1.09	7.0 palm	paralemmin	unknown
Xtevm_656.4.1_XV	2160	7.37	0.58	12.7 follistatin-like3	secreted	unknown
Xtevm_7898.1.1_VX	1042	7.26	1.38	5.3 LOC100495150	unknown	unknown
Xtevm_1491.1.1_VX	920	6.92	0.35	19.8 or56a1l	transmembrane/olfactory receptor	unknown
Xtevm_862.3.1_VX	1238	6.71	0.3	22.4 igsf3	secreted/immunoglobulin	unknown
Xtevm_439.1.1_VV	1035	6.64	0.85	7.8 cmtm5	transmembrane/MARVEL	unknown
Xtevm_189.11.1_VV	1477	6.47	1.14	5.7 bhlhb5	transcription factor/bHLH	unknown
Xtevm_493.3.1_VV	956	6.27	0.87	7.2 unknown	unknown	unknown
Xtevm_170.3.1_VV	3212	6.17	1.15	5.4 cebpa	transcription factor/bZip	unknown
Xtevm_13.19.1_VU	3098	5.77	0.53	10.9 protocadherin9	transmembrane	unknown
Xtevm_227.4.1_VX	512	5.68	0.89	6.4 lypd6	secreted/LY6/PLAUR	unknown
Xtevm_83.30.1_VV	2353	5.49	0.62	8.9 snail2/slug	transcription factor/zinc finger	organizer
Xtevm_163.21.1_VV	835	5.36	0.86	6.2 hoxd9	transcription factor/homeobox	unknown
Xtevm_600.1.1_VV	2732	5.19	0.86	6.0 c4orf31	secreted/fibronectin	unknown
Xtevm_25.13.1_VX	1273	5.04	0.03	168.0 kcng3	transmembrane/potassium channel	unknown

Turquoise, fixed gene models; magenta, known organizer genes; yellow, known dorsal endodermal genes.

Genes, which are splitted into different scaffolds, were manually constructed into single gene models. Those genes are cerberus, siamois, neuropilin 2 and siamois 2 (the same as twin) as follows:

- 1) fixed gene model 1 (cerberus): Xtevm_567.4.1_VX and Xtevm_443.2.1_VV
- 2) fixed gene model 2 (siamois): Xtevm_925.7.1_VX and Xtevm_6099.1.1_VX
- 3) fixed gene model 3 (neuropilin2): Xtevm_208.11.1_VX and Xtevm_208.12.1_VV
- 4) fixed gene model 4 (siamois2/twin): e_gw1.925.59.1

Xtevm_202.16.1_VX and Xtevm_202.17.1_VX were omitted because they are redundant with Xtevm_202.15.1_VV.

Xtevm_68.13.1_VV was omitted because the transcript of Xtevm_68.13.1_VV starts at the + 4 base position from the TSS of Xtevm_68.12.1_VVA (otx2) in the same direction.

Table S4. RNA-seq results for identification of trunk genes.

Xtevm_id	length	rpkm (marginal zone)	rpkm (remaining regions)	marginal zone /remaining region	gene name / synonyms	gene type (structure / family)	expression area in early gastrula
Xtevm_67.42.1_VV	2009	782.22	252.53	3.1	sp5l	transcription factor/zinc finger	ventro-lateral marginal zone
Xtevm_460.2.1_VV	1042	753.61	205.69	3.7	fth1	ferritin	unknown
Xtevm_12.66.1_VXA	2563	664.67	57.70	11.5	vegt	transcription factor/T-box	marginal zone
Xtevm_1051.4.1_VV	728	550.38	107.70	5.1	hes7.2/esr4	transcription factor/bHLH	ventro-lateral marginal zone
Xtevm_432.3.1_VV	2567	517.46	16.47	31.4	t/Xbra	transcription factor/T-box	marginal zone
Xtevm_45.15.1_VV	321	507.47	61.18	8.3	unknown	unknown	unknown
Xtevm_701.3.1_VX	560	379.94	22.71	16.7	LOC100489130	apolipoprotein C	unknown
Xtevm_474.19.1_VV	1884	306.30	46.80	6.5	foxa4	transcription factor/forkhead	dorsal marginal zone
Xtevm_158.14.1_VV	1656	292.70	50.90	5.8	foxd411.2/foxd5.1	transcription factor/forkhead	dorsal marginal zone
fixed gene model5	691	263.54	55.18	4.8	esr5	transcription factor/bHLH	ventro-lateral marginal zone
Xtevm_65.2.1_VV	1494	259.85	25.01	10.4	wnt8a	secreted/Wnt	ventro-lateral marginal zone
fixed gene model6	2464	240.75	44.44	5.4	cyp27c1	CYP hydroxylase	unknown
Xtevm_587.12.1_VV	1673	219.61	25.43	8.6	phlda2	pleckstrin	unknown
Xtevm_31.29.1_VV	2369	216.92	57.75	3.8	zic3	transcription factor/zinc finger	dorsal marginal zone
Xtevm_5.10.1_VV	1135	216.11	2.00	108.1	myf5	transcription factor/bHLH	ventro-lateral marginal zone
Xtevm_40.9.1_VV	2410	211.04	56.99	3.7	znf703	transcription factor/zinc finger	ventro-lateral marginal zone
Xtevm_48.3.1_VV	1913	209.28	43.46	4.8	snai1	transcription factor/zinc finger	marginal zone
Xtevm_91.11.1_VXA	1670	195.68	26.41	7.4	aplnr/msr	transmembrane/receptor	ventro-lateral marginal zone
Xtevm_296.5.1_VV	124	193.70	9.67	20.0	unknown	unknown	unknown
Xtevm_238.5.1_VV	2354	186.95	8.07	23.2	fgf8	secreted/FGF	marginal zone
Xtevm_10.23.1_VV	2327	177.48	9.09	19.5	cdx4	transcription factor/homeobox	ventro-lateral marginal zone
Xtevm_918.1.1_VX	1371	175.12	26.31	6.7	MGC69493	unknown	unknown
Xtevm_494.7.1_VVA	2309	173.81	28.48	6.1	dusp5	phosphatase	dorsal marginal zone
Xtevm_163.16.1_VV	1638	169.93	27.42	6.2	hoxd1	transcription factor/homeobox	ventro-lateral marginal zone
Xtevm_2320.1.1_VX	1436	158.08	18.37	8.6	mix1	transcription factor/homeobox	marginal zone
Xtevm_13.23.1_VV	2274	149.67	27.66	5.4	spry2	signal regulator/sprouty	marginal zone
Xtevm_80.20.1_VV	2056	132.31	11.59	11.4	cdx2	transcription factor/homeobox	ventro-lateral marginal zone
Xtevm_112.14.1_VV	2529	128.25	22.15	5.8	pinhead	transmembrane/coreceptor	ventro-lateral marginal zone
Xtevm_757.1.1_VV	2828	115.95	13.05	8.9	ngfr	transmembrane/receptor	marginal zone
Xtevm_8093.1.1_VX	272	106.13	15.07	7.0	unknown	unknown	unknown
Xtevm_26.19.1_VV	2855	102.93	16.00	6.4	eomes	transcription factor/T-box	marginal zone
Xtevm_940.3.1_VV	1614	101.48	7.13	14.2	gdf3/derriere	secreted/TGFb	marginal zone
Xtevm_22.18.1_VV	1175	99.94	9.94	10.1	mespb	transcription factor/bHLH	ventro-lateral marginal zone
Xtevm_653.1.1_VX	164	99.81	20.36	4.9	unknown	unknown	unknown
Xtevm_3167.1.1_VX	1415	98.55	23.09	4.3	dchr7	dehydrocholesterol reductase	dorsal marginal zone
Xtevm_943.1.1_VV	4083	93.71	13.50	6.9	pcdh8.2/papc	transmembrane/protocadherin	dorsal marginal zone
Xtevm_55.9.1_VV	403	90.39	23.02	3.9	hes6.1	transcription factor/bHLH	ventro-lateral marginal zone
Xtevm_8.8.1_VV	4388	89.42	10.17	8.8	flrt3	transmembrane/fibronectin	marginal zone
Xtevm_292.10.1_VV	2504	86.32	5.68	15.2	pygm	phosphorylase	unknown
Xtevm_261.4.1_VV	4035	85.27	12.43	6.9	plk3	kinase	unknown
Xtevm_481.1.1_VX	1480	83.89	21.27	3.9	cyp2c8.1	CYP hydroxylase	unknown
Xtevm_432.4.1_VV	1820	82.51	0.43	191.9	t2/Xbra2	transcription factor/T-box	marginal zone
fixed gene model7	2647	79.49	9.98	8.0	wnt11	secreted/Wnt	marginal zone
Xtevm_36.5.1_VV	4350	78.05	19.17	4.1	prickle1	signal regulator/prickle	marginal zone
Xtevm_91.22.1_VX	4121	75.92	19.99	3.8	slc12a3.2	transmembrane/sodium chloride transpoter	unknown
Xtevm_704.1.1_VV	2518	75.45	6.71	11.2	spry1	signal regulator/sprouty	marginal zone
Xtevm_1021.1.1_VX	855	74.35	16.91	4.4	slc43a1	transmembrane/carrier	unknown
Xtevm_968.1.1_VV	1350	73.31	0.79	92.8	fgf20	secreted/FGF	marginal zone
Xtevm_852.2.1_VX	1945	73.23	18.80	3.9	cntm8	transmembrane/MARVEL	unknown
Xtevm_2.66.1_VV	2954	71.35	16.02	4.5	dll1	transmembrane/delta	marginal zone
fixed gene model8	1312	70.42	2.44	28.8	lefty	secreted/TGFb	dorsal marginal zone
Xtevm_107.16.1_VV	1662	67.34	14.18	4.7	rasl11b	GTPase/ras	ventro-lateral marginal zone
Xtevm_73.38.1_VV	1073	66.91	12.85	5.2	unknown	transcription factor/bHLH	unknown
Xtevm_100.7.1_VV	2054	65.36	18.23	3.6	rhob	GTPase/rho	dorsal marginal zone
Xtevm_73.39.1_VV	1327	64.19	6.35	10.1	unknown	transcription factor/bHLH	unknown
Xtevm_224.20.1_VV	3970	63.02	9.62	6.6	epha4	transmembrane/receptor	marginal zone
Xtevm_1440.1.1_VX	1862	63.00	15.29	4.1	alpl	phosphatase	unknown
Xtevm_10.30.1_VV	1261	62.22	17.59	3.5	zc4h2	transcription factor/zinc finger	unknown
fixed gene model9	1260	60.95	2.46	24.8	tbx6	transcription factor/T-box	ventro-lateral marginal zone
Xtevm_11.31.1_VV	3980	60.73	17.25	3.5	pkfb3	phosphatase	unknown
Xtevm_648.3.1_VV	2832	59.20	15.08	3.9	kcnk6	potassium phannel	marginal zone
Xtevm_418.11.1_VX	2085	58.86	13.08	4.5	c9	complement component	dorsal ectoderm
Xtevm_481.2.1_VV	2213	56.04	14.26	3.9	LOC100145695	CYP hydroxylase	unknown
Xtevm_305.4.1_VV	2678	54.61	14.62	3.7	rab34	GTPase/rab	unknown
Xtevm_131.7.1_VU	1843	52.52	10.85	4.8	LOC100495801	unknown	unknown
Xtevm_4.44.1_VV	1708	47.76	2.40	19.9	foxd3	transcription factor/forkhead	dorsal marginal zone
Xtevm_427.11.1_VX	3429	47.43	3.91	12.1	ca14	carbonic anhydrase	unknown
Xtevm_132.1.1_VV	4087	46.79	10.48	4.5	ephb3	transmembrane/receptor	unknown
Xtevm_100.13.1_VV	1011	45.75	2.38	19.2	mesogenin1	transcription factor/bHLH	ventro-lateral marginal zone
Xtevm_763.1.1_VV	3108	44.92	14.86	3.0	has2	hyaluronan synthase	marginal zone
Xtevm_783.19.1_VV	4305	44.86	10.86	4.1	cingulin	cell adhesion molecule	unknown
Xtevm_719.5.1_VX	1315	44.51	5.66	7.9	bix1.2	transcription factor/homeobox	unknown
Xtevm_164.2.1_VV	2808	43.07	5.29	8.1	adamts1	metallopeptidase	dorsal marginal zone
fixed gene model10	3371	42.98	2.34	18.4	foxb1	transcription factor/forkhead	dorsal ectoderm

Table S4 (continued)

Xtevm_375.8.1_VV	2907	42.20	13.48	3.1tpbg/waif1a2	glycoprotein	unknown
Xtevm_281.18.1_VV	1040	41.52	5.92	7.0prf5l	proline rich protein	unknown
Xtevm_493.6.1_VX	1208	40.68	12.34	3.3mypo	transcription factor/myb	unknown
Xtevm_232.1.1_VV	4812	37.75	6.35	5.9zeb2/zfhx1b/sip1	transcription factor/homeobox	dorsal marginal zone
Xtevm_493.2.1_VV	1688	37.60	6.34	5.9pcbp2	polyC-binding	unknown
Xtevm_98.11.1_VV	8585	37.34	9.91	3.8akap2	scaffold protein	dorsal marginal zone
Xtevm_482.11.1_VV	4131	37.32	3.93	9.5ssh2	phosphatase	unknown
Xtevm_250.12.1_VX	826	36.29	5.13	7.1wisp3	secreted/CTGF	unknown
Xtevm_781.2.1_VV	1775	36.28	8.37	4.3rbm38/seb4r	RNA-binding protein	ventro-lateral marginal zone
Xtevm_998.1.1_VX	118	35.42	1.86	19.0unknown	unknown	unknown
Xtevm_46.4.1_VX	1236	35.04	4.15	8.4fam134b	unknown	unknown
Xtevm_158.13.1_VX	1016	33.18	0.91	36.5foxd411.2/foxd5.2	transcription factor/forkhead	dorsal marginal zone
fixed gene model11	2619	33.14	7.00	4.7esr10	transcription factor/bHLH	ventro-lateral marginal zone
Xtevm_719.3.1_VV	1166	32.92	7.00	4.7mixer	transcription factor/homeobox	marginal zone
Xtevm_95.18.1_VV	2370	32.86	3.21	10.2foxc1	transcription factor/forkhead	dorsal ectoderm
Xtevm_38.40.1_VV	1585	32.69	5.40	6.1st3gal2.2	sialyltransferase	unknown
Xtevm_107.13.1_VV	5746	32.50	9.72	3.3dlc1/rhogap7	rhogap	unknown
Xtevm_493.3.1_VV	956	30.98	3.26	9.5unknown	unknown	unknown
Xtevm_819.2.1_VV	1123	30.30	7.15	4.2unknown	unknown	unknown
Xtevm_163.17.1_VV	969	29.92	5.47	5.5unknown	unknown	unknown
Xtevm_233.12.1_VV	2897	29.69	5.88	5.0MGC89906	unknown	unknown
Xtevm_319.9.1_VV	1958	28.07	2.07	13.6kng1	kininogen	unknown
Xtevm_370.5.1_VV	2503	28.01	1.31	21.4plod2	lysyl hydroxylase	unknown
Xtevm_233.10.1_VV	1628	27.73	5.64	4.9fam124a	unknown	unknown
Xtevm_267.7.1_VV	825	27.49	1.78	15.4sebox	transcription factor/homeobox	unknown
fixed gene model12	3920	27.39	3.47	7.9scube2	secreted/CUB,EGF-like	unknown
fixed gene model13	2330	27.07	2.67	10.1rbm20	RNA-binding protein	unknown
Xtevm_376.5.1_VV	1179	27.04	8.43	3.2plekho1	pleckstrin	unknown
Xtevm_54.12.1_VV	3206	26.64	8.64	3.1sh3rf1/posh	E3 ubiquitin ligase	marginal zone
Xtevm_181.9.1_VV	2441	26.40	4.57	5.8foxc2	transcription factor/forkhead	unknown
Xtevm_45.38.1_VV	6170	26.39	5.83	4.5lamb1	secreted/extracellular matrix	unknown
Xtevm_555.6.1_VX	2807	26.08	0.17	153.4tmprss9	protease	unknown
Xtevm_3157.1.1_VX	96	25.41	8.33	3.1nipal2	NIPA-like domain	unknown
Xtevm_432.2.1_VV	725	24.53	2.52	9.7unknown	unknown	unknown
Xtevm_112.12.1_VV	958	23.16	3.86	6.0tspan3	integral membrane protein	unknown
Xtevm_507.2.1_VV	1941	22.55	1.81	12.5LOC779592	ceramid kinase	unknown
Xtevm_330.7.1_VV	3066	20.22	3.68	5.5enpp2	pyrophosphatase/phosphodiesterase	unknown
Xtevm_305.1.1_VX	2221	19.96	3.93	5.1traf4	signal regulator/TRAF	marginal zone
Xtevm_84.2.1_VV	3157	19.04	4.55	4.2cadherin2/N-cadherin	transmembrane/cadherin	unknown
Xtevm_533.9.1_VU	1942	18.59	4.29	4.3tmem45a	transmembrane	unknown
Xtevm_12.98.1_VX	3513	18.57	3.86	4.8mmp17	metallopeptidase	unknown
Xtevm_692.2.1_VU	2013	17.87	5.56	3.2follistatin-like1	secreted	unknown
fixed gene model14	1774	17.75	0.57	31.1myod1	transcription factor/bHLH	ventro-lateral marginal zone
fixed gene model15	4050	17.53	3.14	5.6sphk2	kinase	unknown
Xtevm_559.3.1_VV	1766	16.92	3.48	4.9cdx1	transcription factor/homeobox	ventro-lateral marginal zone
Xtevm_37.14.1_VX	1381	15.45	0.35	44.1actc1	actin	unknown
fixed gene model16	1895	14.68	3.35	4.4LOC100492804	unknown	unknown
Xtevm_325.1.1_VU	2062	14.08	3.70	3.8btg5/xbtg-x	anti-proliferation factor	dorsal marginal zone
Xtevm_409.13.1_VV	1881	13.09	2.93	4.5numbl	signal regulator/numb	unknown
Xtevm_44.25.1_VU	1549	12.77	3.05	4.2nuak1	kinase	marginal zone
Xtevm_10.54.1_VV	606	12.68	0.66	19.2fgf16	secreted/FGF	unknown
Xtevm_996.7.1_VV	484	12.66	2.29	5.5LOC100492750	unknown	unknown
Xtevm_6.67.1_VX	877	12.22	0.91	13.4slc9a5	transmembrane/cation proton antipoter	unknown
Xtevm_255.4.1_VV	2125	12.20	2.22	5.5wdr16	WD repeat	unknown
Xtevm_37.27.1_VV	2575	11.27	1.40	8.1spred1	signal regulator/spred	dorsal marginal zone
Xtevm_34.39.1_VX	1400	11.17	1.62	6.9nodal2/Xnr2	secreted/TGFb	dorsal marginal zone
Xtevm_613.1.1_VV	813	10.77	1.80	6.0rspo3	secreted/R-spondin	dorsal marginal zone
Xtevm_73.36.1_VV	409	10.75	3.37	3.2hes8	transcription factor/bHLH	unknown
Xtevm_56.16.1_VV	2005	10.13	1.37	7.4hoxa1	transcription factor/homeobox	ventro-lateral marginal zone
Xtevm_204.7.1_VV	1194	9.68	1.38	7.0nodal4/Xnr4	secreted/TGFb	dorsal marginal zone
Xtevm_356.14.1_VV	1726	9.43	2.92	3.2dnaaf3	dynein axonemal assembly factor	unknown
Xtevm_28.18.1_VX	559	9.37	0.39	24.0shh	secreted/hedgehog	dorsal marginal zone
Xtevm_60.5.1_VV	2710	9.19	1.87	4.9fhdc1	FH2 domain	unknown
Xtevm_105.25.1_VU	1196	9.14	1.60	5.7C2orf77-like	unknown	unknown
Xtevm_30.55.1_VV	1554	9.09	1.06	8.6sfrp1	secreted/sfrp	dorsal marginal zone
Xtevm_1104.2.1_VU	421	9.07	2.32	3.9tshb	secreted/hormone	unknown
Xtevm_282.12.1_VX	538	8.99	0.14	64.2npb	neuropeptide	unknown
Xtevm_22.24.1_VV	945	8.98	1.13	7.9c15orf26	unknown	unknown
Xtevm_630.7.1_VVA	3015	8.95	2.11	4.2frmd4b	FERM domain	unknown
Xtevm_1605.1.1_VX	928	8.86	1.39	6.4unknown	unknown	unknown
Xtevm_362.5.1_VU	607	8.86	1.91	4.6btnl2l	butyrophilin	unknown
Xtevm_194.2.1_VV	1721	8.78	1.60	5.5tek2	cytstoskeletal protein	unknown
Xtevm_483.7.1_VX	2413	8.57	2.47	3.5mtor	kinase	unknown
Xtevm_198.23.1_VU	2435	8.51	2.27	3.7adam33	metallopeptidase	marginal zone
Xtevm_22.17.1_VV	1518	8.49	2.23	3.8mespa	transcription factor/bHLH	ventro-lateral marginal zone
Xtevm_107.26.1_VV	3388	8.44	2.56	3.3pdgfra	transmembrane/receptor	ventro-lateral marginal zone

Table S4 (continued)

Xtevm_448.2.1_VV	2250	7.92	2.57	3.1 ccdc151	coiled-coil	unknown
Xtevm_11.8.1_VV	304	7.89	0.85	9.3 LOC100491987	unknown	unknown
Xtevm_47.4.1_VV	1075	7.81	1.45	5.4 Enkur	enkurin	unknown
Xtevm_792.2.1_VV	3912	7.65	1.34	5.7 mmp14	metallopeptidase	unknown
Xtevm_55.52.1_VV	1478	7.58	0.96	7.9 daw1	dynein assembly factor	unknown
Xtevm_56.14.1_VV	539	7.49	0.48	15.6 unknown	unknown	unknown
Xtevm_972.2.1_VV	1474	7.42	0.44	16.9 unknown	unknown	unknown
Xtevm_1023.5.1_VX	1084	7.29	1.89	3.9 rsph1	ciliary protein	unknown
Xtevm_565.7.1_VX	2056	7.05	1.41	5.0 c7	complement component	unknown
Xtevm_149.15.1_VX	525	7.02	0.41	17.1 emr1	hormone receptor-like	unknown
Xtevm_871.1.1_VX	1406	6.99	0.72	9.7 fam55c-like	unknown	unknown
Xtevm_338.8.1_VX	703	6.77	0.49	13.8 arl3.2	ADP-ribosylation factor	unknown
Xtevm_168.6.1_XV	1508	6.66	0.55	12.1 LOC100498211	unknown	unknown
Xtevm_238.7.1_VV	723	6.58	1.72	3.8 fbxw4	F-box, WD repeat	unknown
Xtevm_828.4.1_VX	2111	6.53	0.54	12.1 kremen2	transmembrane/receptor	ventro-lateral marginal zone
Xtevm_17.13.1_VV	898	6.48	1.58	4.1 srrd	SRR1 domain	unknown
Xtevm_197.11.1_VV	1662	6.39	1.17	5.5 LOC100158459	unknown	unknown
Xtevm_968.2.1_XV	1372	6.38	0.22	29.0 micu3	Ca2+ binding	unknown
Xtevm_280.17.1_VU	1166	6.25	0.95	6.6 st3gal5	sialyltransferase	unknown
Xtevm_350.9.1_VU	4044	6.19	1.76	3.5 egflam/pikachurin	secreted/extracellular matrix	unknown
Xtevm_499.2.1_VV	754	6.07	0.29	20.9 spry4	signal regulator/sprouty	unknown
Xtevm_485.5.1_VU	4105	5.86	0.26	22.5 gli1	transcription factor/zinc finger	unknown
Xtevm_279.12.1_VV	1804	5.84	1.77	3.3 ccdc19	coiled-coil	unknown
Xtevm_389.39.1_VV	2345	5.76	1.40	4.1 unknown	unknown	unknown
Xtevm_1845.2.1_VV	449	5.74	1.29	4.4 unknown	unknown	unknown
Xtevm_213.12.1_VV	4169	5.65	1.17	4.8 sall3	transcription factor/zinc finger	unknown
Xtevm_735.12.1_VV	1459	5.48	0.51	10.7 lrcc23	leucine rich repeat	unknown
Xtevm_47.15.1_VV	1694	5.46	0.65	8.4 spag6	unknown	unknown
Xtevm_244.2.1_VV	2921	5.45	1.35	4.0 stox1	transcription factor/storkhead	unknown
Xtevm_1202.2.1_VX	1831	5.34	0.85	6.3 ckap4	cytoskeleton associated protein	unknown
Xtevm_1014.5.1_VV	794	5.03	1.62	3.1 dnajb13	DnaJ/Hsp40	unknown

Turquoise, fixed gene models; orange, known marginal genes; yellow, known dorsal marginal genes; blue, known ventro-lateral marginal genes; light blue, known dorsal ectodermal genes.

Genes, which are splitted into different scaffolds, were manually constructed into single gene models. Those genes are *esr5*, *cyp27c1*, *wnt11*, *lefty*, *tbx6*, *foxb1*, *esr10*, *scube2*, *rbm20*, *myod1*, *sphk2* and *LOC100492804* as follows:

- 1) fixed gene model 5 (*esr5*): Xtevm_1051.6.1_VX and Xtevm_1051.7.1_VV
- 2) fixed gene model 6 (*cyp27c1*): Xtevm_893.5.1_VV, Xtevm_893.6.1_VV and Xtevm_4119.1.1_VX
- 3) fixed gene model 7 (*wnt11*): Xtevm_117.9.1_VV and Xtevm_117.10.1_VV
- 4) fixed gene model 8 (*lefty*): Xtevm_719.12.1_VX and Xtevm_719.13.1_VV
- 5) fixed gene model 9 (*tbx6*): Xtevm_1489.5.1_VX and Xtevm_1655.2.1_VV
- 6) fixed gene model 10 (*foxb1*): Xtevm_324.12.1_VV and Xtevm_324.13.1_VV
- 7) fixed gene model 11 (*esr10*): Xtevm_73.37.1_VVA and Xtevm_11537.1.1_VV
- 8) fixed gene model 12 (*scube2*): Xtevm_1116.2.1_VV and Xtevm_1184.1.1_VX
- 9) fixed gene model 13 (*rbm20*): Xtevm_1137.4.1_VV and Xtevm_1137.5.1_VX
- 10) fixed gene model 14 (*myod1*): Xtevm_2758.1.1_VV and Xtevm_2758.2.1_VV
- 11) fixed gene model 15 (*sphk2*): Xtevm_356.15.1_VU, Xtevm_356.16.1_VV and Xtevm_356.19.1_VV
- 12) fixed gene model 16 (*LOC100492804*): Xtevm_556.4.1_VU and Xtevm_556.5.1_VVA

Xtevm_587.13.1_VV was omitted because it is redundant with Xtevm_587.12.1_VV.

Xtevm_1670.1.1_VX (*ilk*), Xtevm_1035.2.1_VX (*mfap2*), Xtevm_8544.1.1_VX (*prpf19*) and Xtevm_4643.1.1_VX (novel protein containing P21-Rho-binding domain) were omitted because they are redundant with genes which were not enriched in the marginal region.

Xtevm_6278.1.1_VX was omitted because it is redundant with a ribosomal gene.

Table S5. Primer sets (5' to 3') for ChIP-qPCR

target	forward primer	reverse primer	amplicon size (bp)	references
<i>acta1</i> -promoter	TCCGTGGGCTTGGTATTATC	AGAGGCTGGTGCCGTAAAAG	74	this study
<i>bambi</i> -U2	TGGGCTTTTGTCTGCTAAG	CTTTGTTGAGCAAGGAAGG	130	this study
<i>cerberus</i> -U1	ATCAGGCTTGACAGAAATC	AACTGCAGTACTTATGGGAGGAC	143	this study
<i>cerberus</i> -U2	CCTGCTAATTTGGCTCAACC	AAAGGCTTCACCAGCAAGAG	130	this study
<i>chordin</i> -D1	GGAGACAGATTTGGCTGCTA	CAATCCAGAACTTCCTCTTCA	103	this study
<i>chordin</i> -U3	CACATGCATCTTTGCTCCTCAG	AAGCTAAACTGGGCCATCAG	82	this study
<i>crescent</i> -U2	GAGGCACTGTGAGCCAAAC	TTCCCAGGGAGTGTGAGATAAC	127	this study
<i>ef1α</i> -promoter	TCCTGTTTAGCGTTGCGTTC	ACTCAGCCTCCATCTGTGG	101	this study
<i>fgf8</i> -U2	AGCCGATTGTCCCAATACAC	ACAGGTACACTGCACCCAAG	80	this study
<i>frizzled8</i> -U6	TGTCCTAACCAAGCGCAAATG	TGCTCACCCACCTTAAATG	79	this study
<i>gsc</i> -U1	AATGACAGCCAACAGCTCAAGGGACA	TGGCAGACTCTCCCTGTACTTATTCAGA	91	Blythe et al., 2009
<i>hhex</i> -D3	CAGCAGTTGTCCAAGAAACC	TGCATTACAGCGTACGTTCC	121	this study
<i>lim1</i> -U10	CTGAACGTGTTCGATTGTG	CACTTGCACTTGACAGAAAGG	79	this study
<i>msgn1</i> -U1	TCACTCGGAACCTTGTGAGC	TTTACCAGTGGATGGTGCAG	143	this study
<i>msx1</i> -U2	GAAAAGGACCGGTGTGAATG	TAATGGGCAGACAAGGGATG	141	this study
<i>msx2</i> -U2	GTAAATTCGGTGGCACCTTG	TTGAAGTCAGACACCGCTACC	134	this study
<i>nkx6.2</i> -D1	AGGCATAAAGGCAACACTGG	ATGATTGCTGTGGGGAAGT	146	this study
<i>noggin</i> -D1	GGGCTGACACCTTTTGAATG	CTGGGGTTTCTGAATGAAG	136	this study
<i>not</i> -U2	ATACTGGGCCCCACTTCACA	TCTTGTGGCGGATGAAAGA	71	this study
<i>otx2</i> -U1	GGGGAAGACCTAACACACCA	ATTCAGCATTCGAAGCCCTA	97	this study
<i>otx5</i> -U1	TCAACCCTCGAATGCTTCTC	AACCCCATGAGTGTCCATTG	109	this study
<i>ror2</i> -D1	CTCCCAGGGAGAGTTAAATGG	AAGCAGTGAGGTTGCATATTGG	101	this study
<i>smad7</i> -D2	TGCCTAGAAGAATGCCAGTG	TCCATGAGGAAAGGGAGAAG	77	this study
<i>szl</i> -U2	AAGTGAGATGGCATCGTTCC	AGCAACAGGGCAATTAGCTG	120	this study
<i>ventx1.1</i> -U3	TGGGAGCAGAGGTACAAACAG	TTCCCTGAATAGGAGCCTTC	71	this study
<i>ventx1.2</i> -U1	TCCAAATTGCCCTCATGG	CTGTGCACATGGTTAAGTTGC	106	this study
<i>ventx2.1</i> -U2	CCATTGTCCCTCAGCAAAAC	GGGCTTTCCAAACCCATAAC	135	this study
<i>wnt11</i> -D1	GCAGCAGCCAGAGAATCTATC	ACTTGTAAACGGGGTTGTTC	134	this study
<i>wnt8a</i> -U1	CCAAAGCTGTGATCGCCTAT	CCAAAGGGAAGCAACTCAA	73	this study

Table S6. Primer sets (5' to 3') for RT-qPCR

species	gene	forward primer	reverse primer	amplicon size (bp)	references
<i>X. tropicalis</i>	<i>ef1α</i>	TGATCCCAGGAAAGCCTATG	CATGTCACGGACAGCAAAAC	77	this study
	<i>brachyury</i>	GCTCACCAATGAGATGATCG	TACATGGCATTGGGATCCAG	93	this study
	<i>cerberus</i>	TTCTGCCTTGTGAATGATGG	TCCCATTCCTTTCTTTTC	108	this study
	<i>gsc</i>	TAAACCCAGCGCTAAGGAAC	AACATGCCAGAAGGCATCAC	78	this study
	<i>lhx1</i>	GCGAGTGAAGTGCAATCTGAC	TACCAAAGCGCCTGAAGAAG	87	this study
	<i>not</i>	CCTGTCTTTCCTGCATTGG	AAGAAGCTTTGGGGTCCCTG	88	this study
	<i>otx2</i>	AAATCAACTTGCCCGAGTCC	ATTCTGCTGCTGCTGTTGC	86	this study
	<i>szl</i>	AAACAAGTCTGCTCCTTCC	CTTGCCATGATGGATGTCAC	138	this study
<i>wnt8a</i>	ATCTGGAAGTTGCAGTGTGC	TCGTGCTTGATCTTCAGGTG	84	this study	
<i>X. laevis</i>	<i>ef1α</i>	CTACAAATGTGGTGGCATCG	GCTCTGCCTTCAGTTTGTC	116	this study
	<i>actc1</i> (3'UTR)	TACACACACTCCTCCAAGCAAC	GTGACTGTCCAGGTCTTGTTTTTC	70	this study
	<i>bracyury</i>	ATGGTGGAGGCCAGATTATG	TCATTCTGGTATGCGGTCAC	145	this study
	<i>cerberus</i>	TGCATCTCTCCATGTTCC	AATFCAGCGTCAGGTGGTTC	97	this study
	<i>chordin</i>	TCTGCACGATATGAGCAAGG	TCGGGCATTAGCATCTATCC	70	this study
	<i>gsc</i> (3'UTR)	CGATGTGGGATCTGTACATAGAGTG	CAGTGTATCTGTGCAAAATGTCTG	92	this study

Figure S1 (Yasuoka et al.)

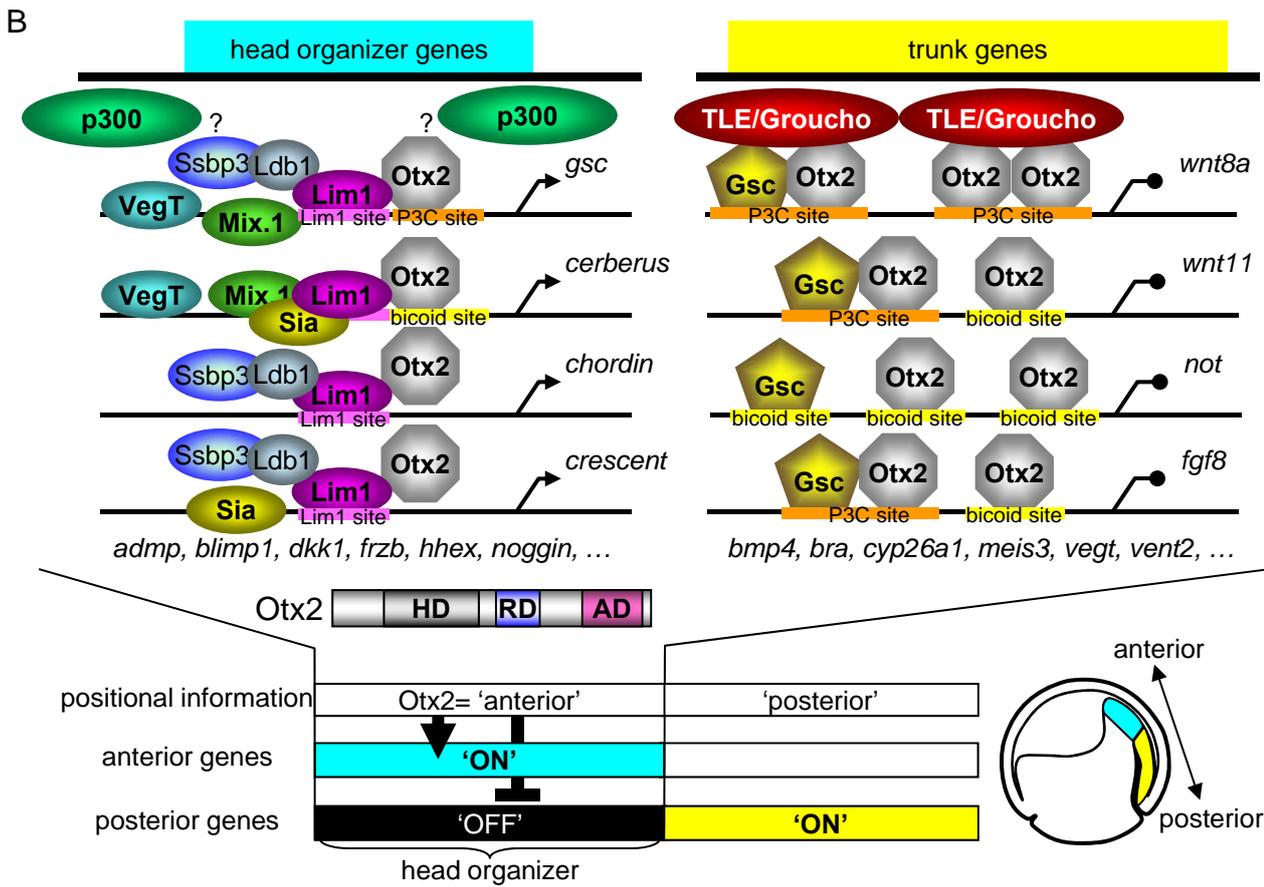
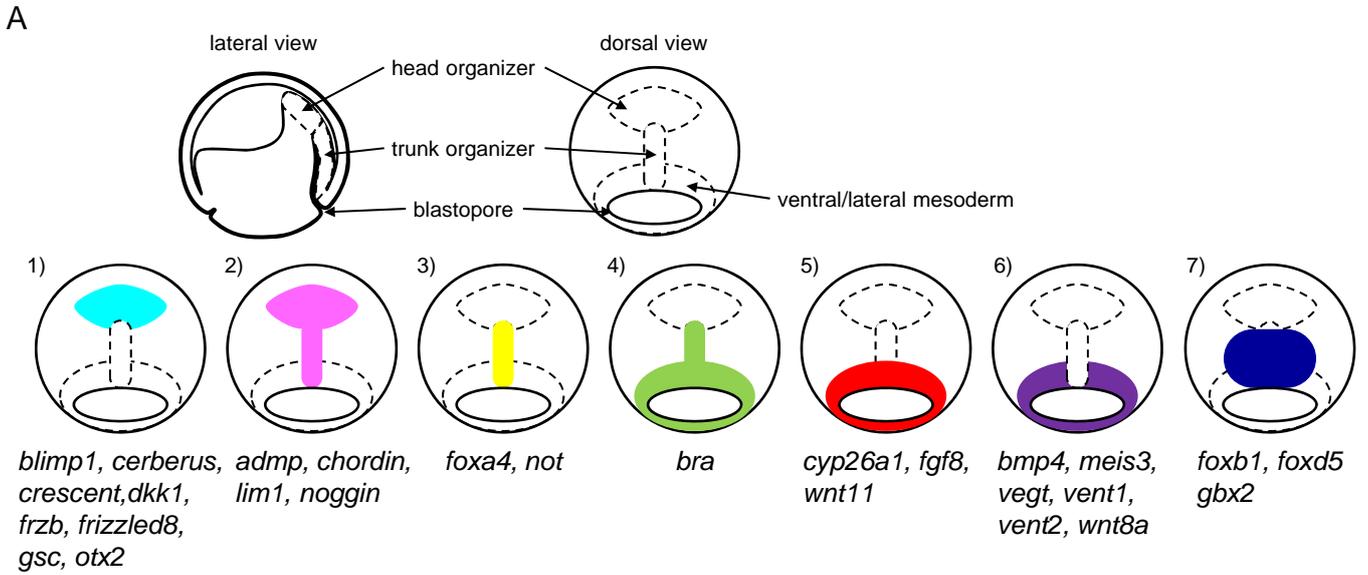
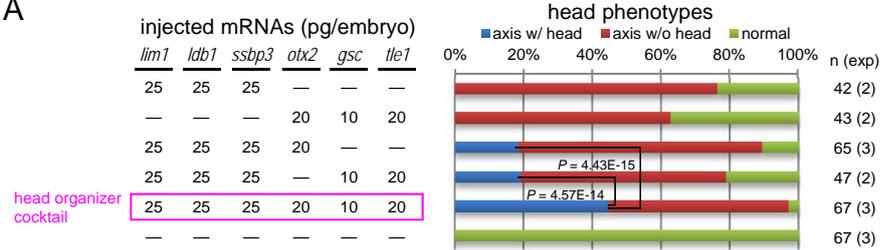


Figure S3 (Yasuoka et al.)

A



B

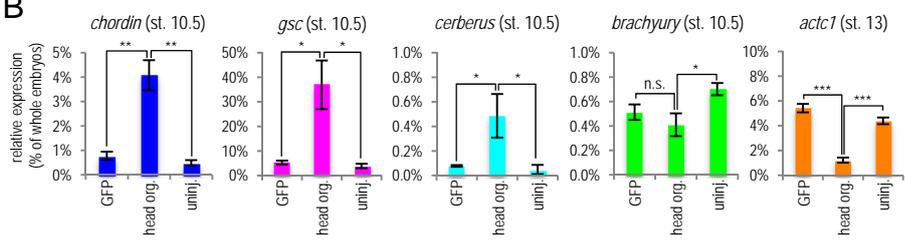


Figure S4 (Yasuoka et al.)

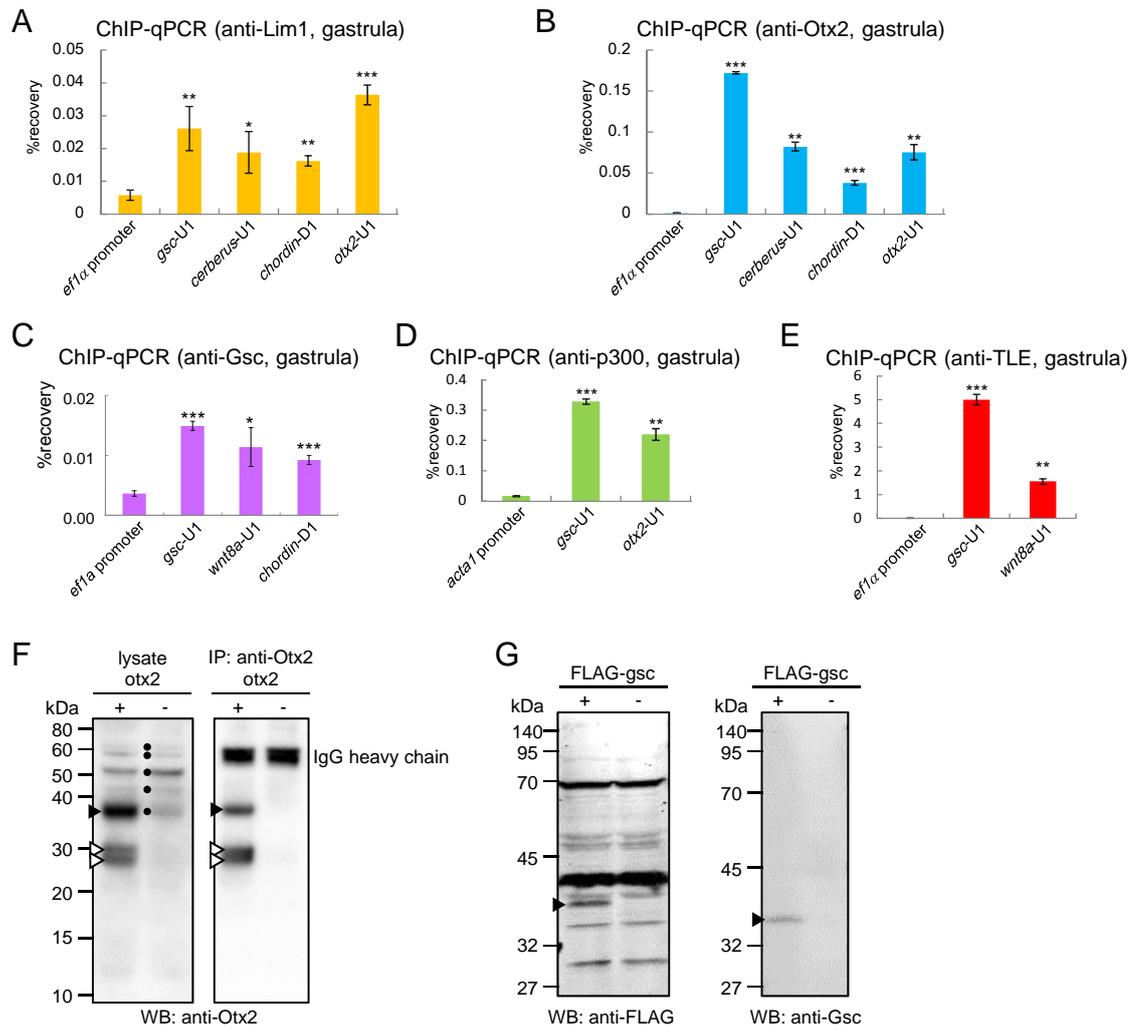
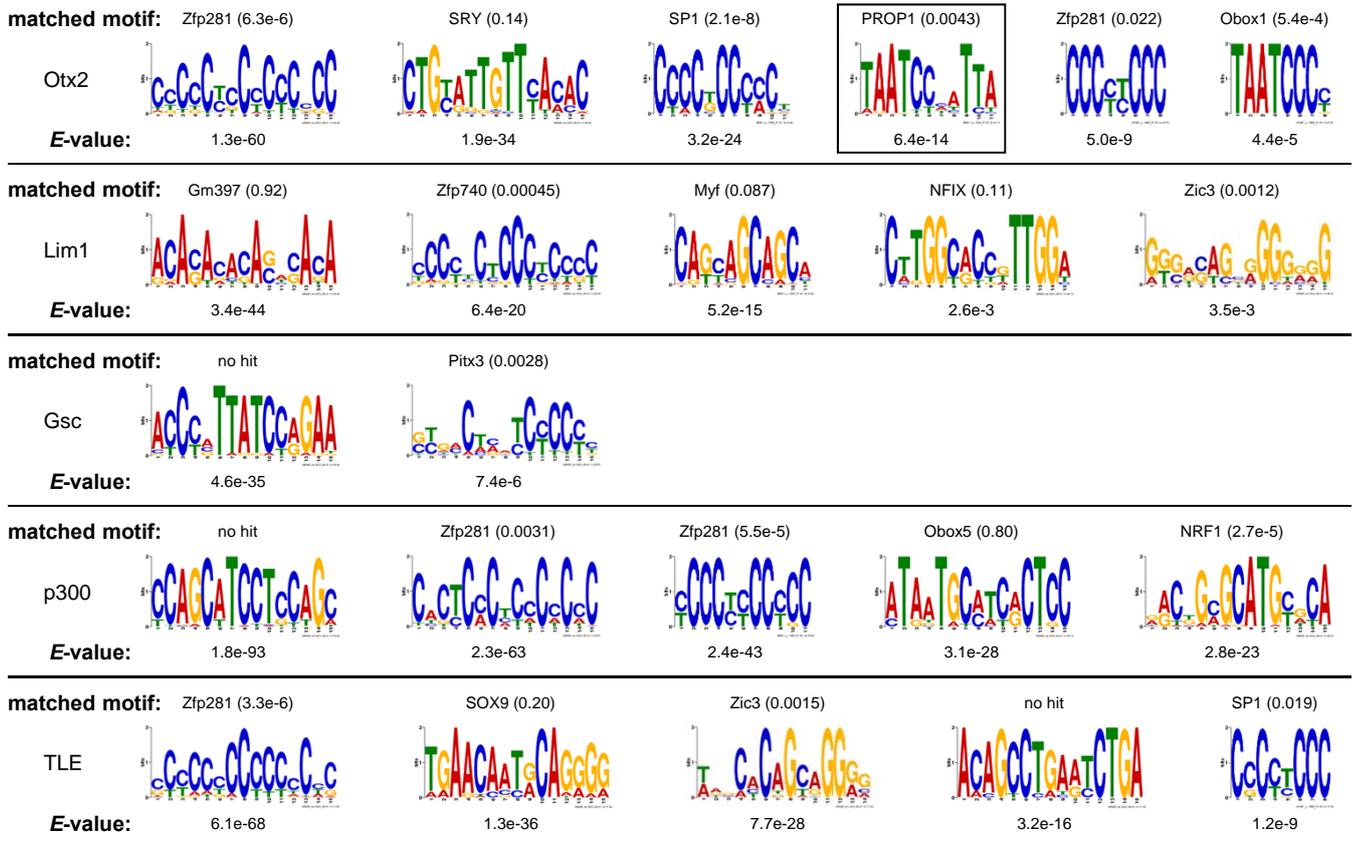


Figure S5 (Yasuoka et al.)

A Motifs discovered by MEME



B Motifs discovered by DREME

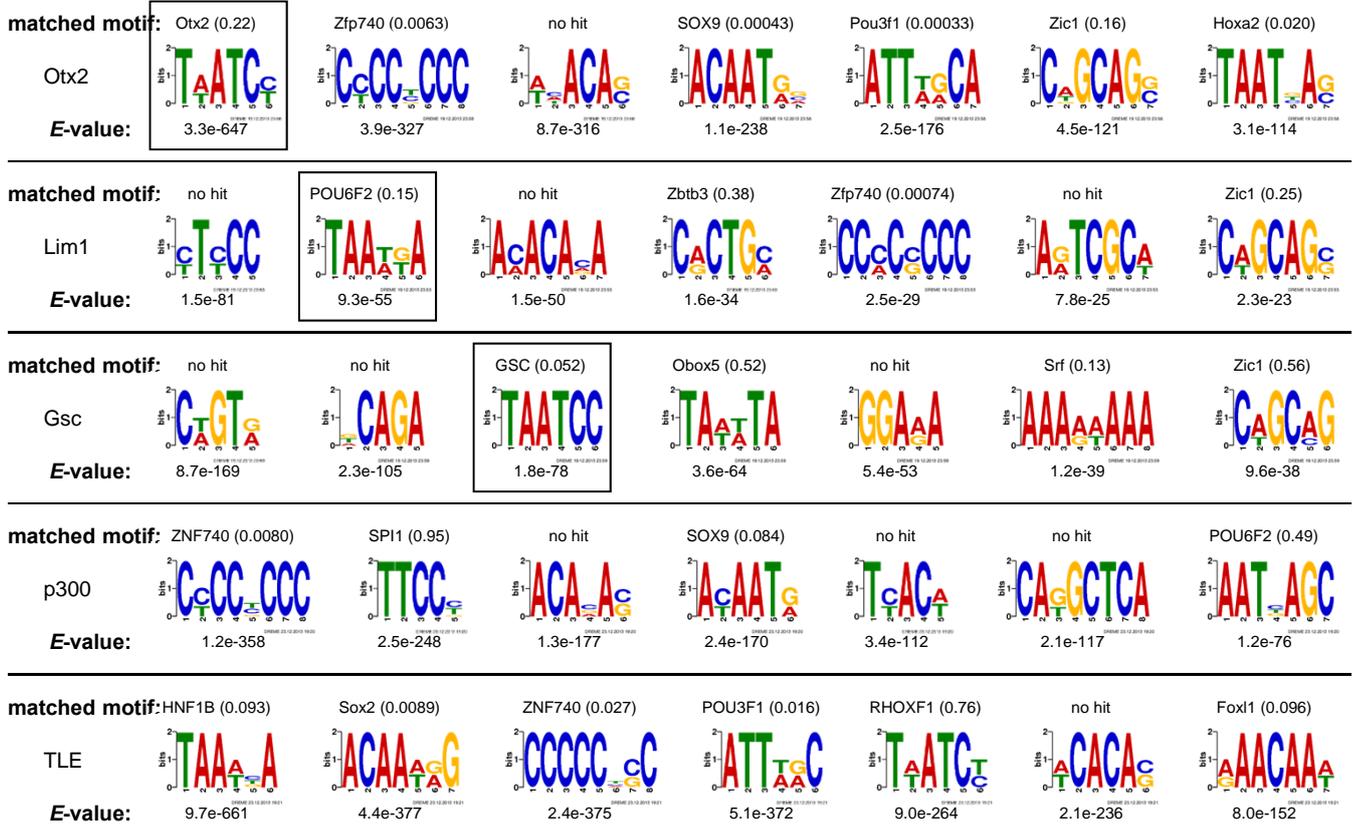


Figure S6 (Yasuoka et al.)

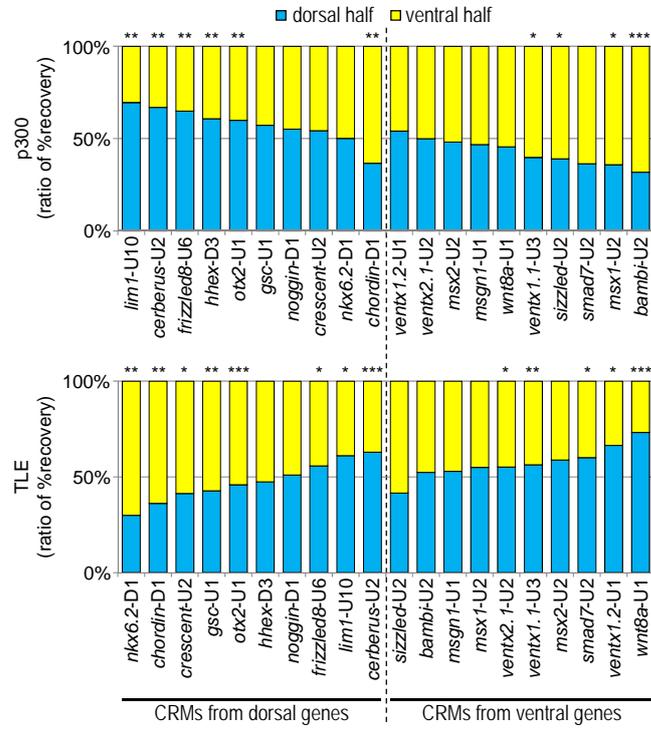


Figure S7 (Yasuoka et al.)

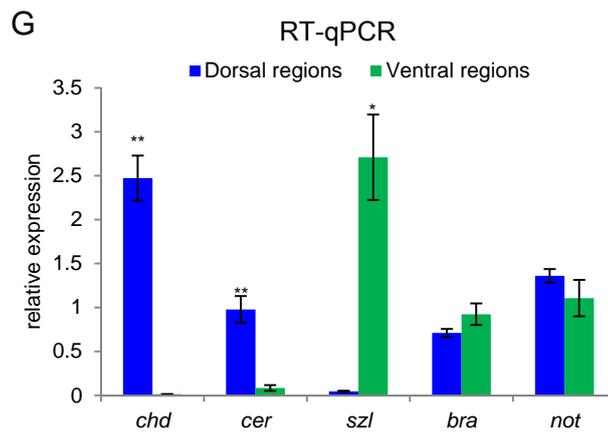
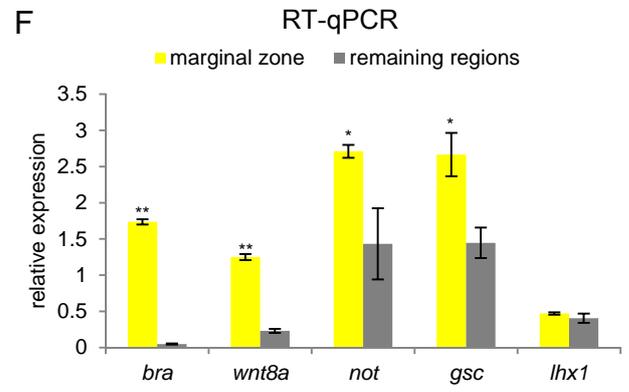
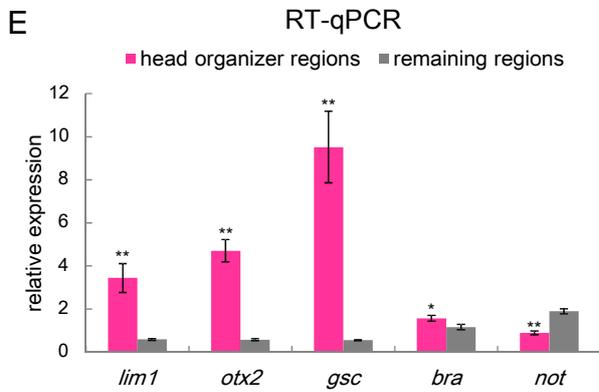
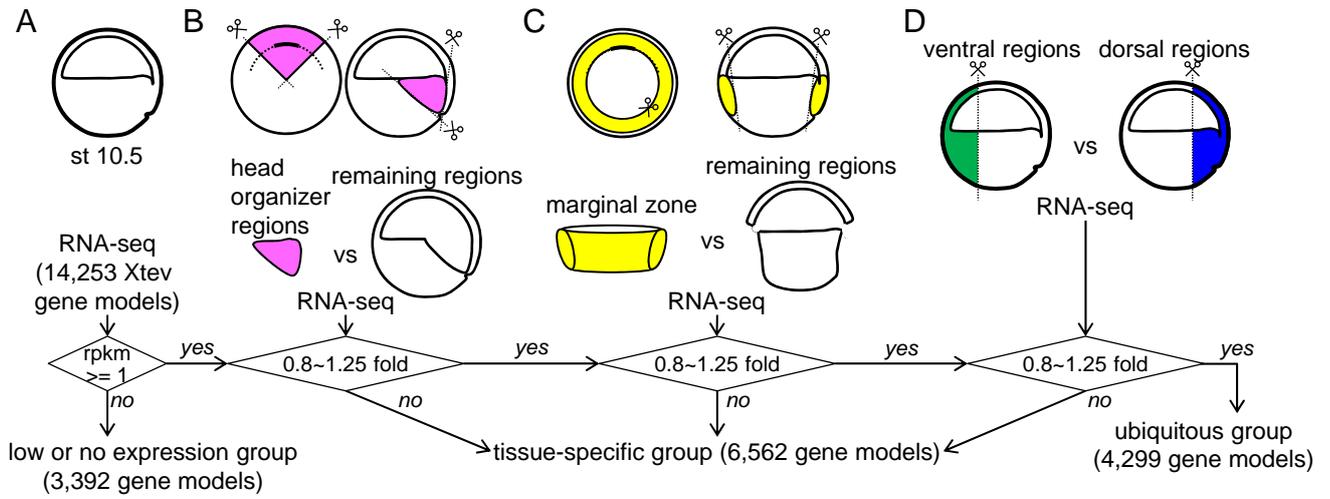


Figure S8 (Yasuoka et al.)

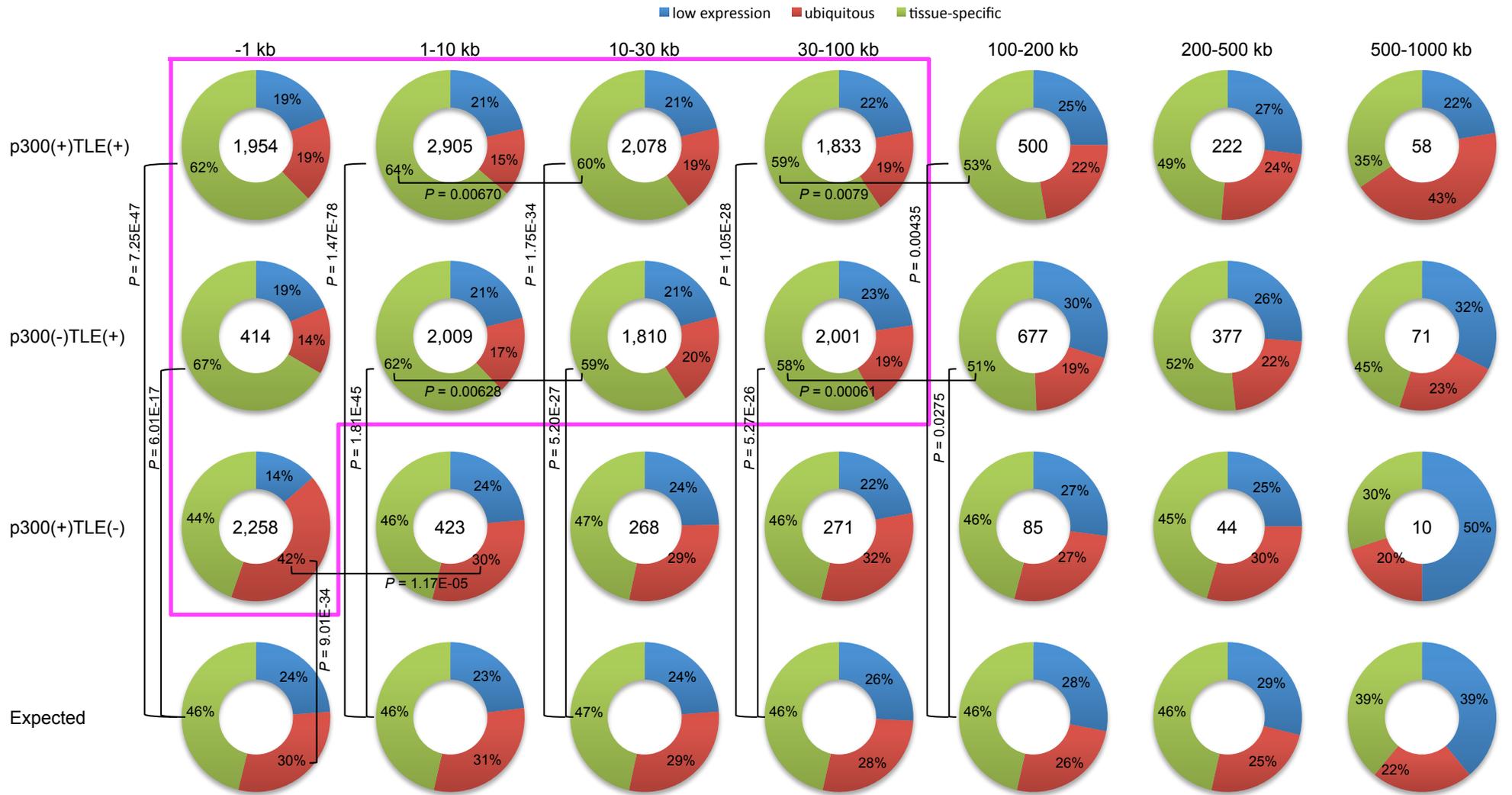


Figure S9 (Yasuoka et al.)

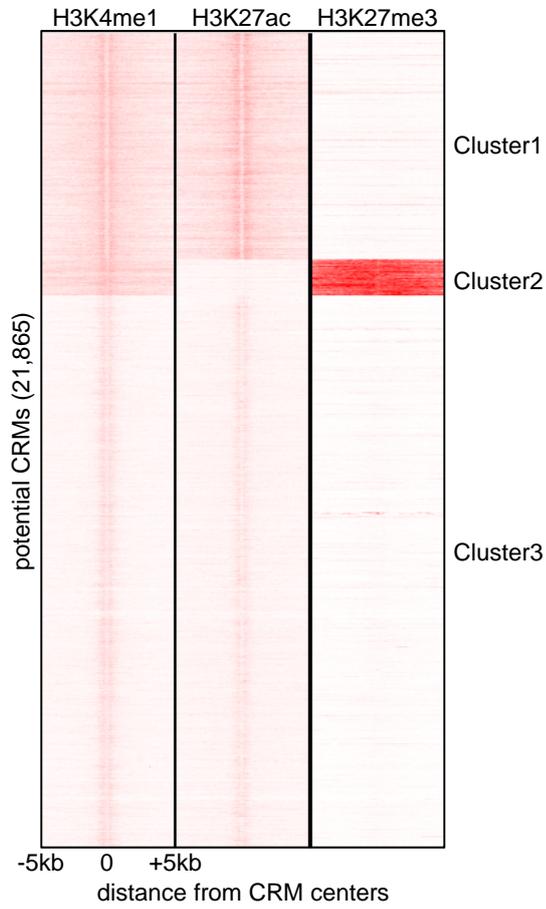
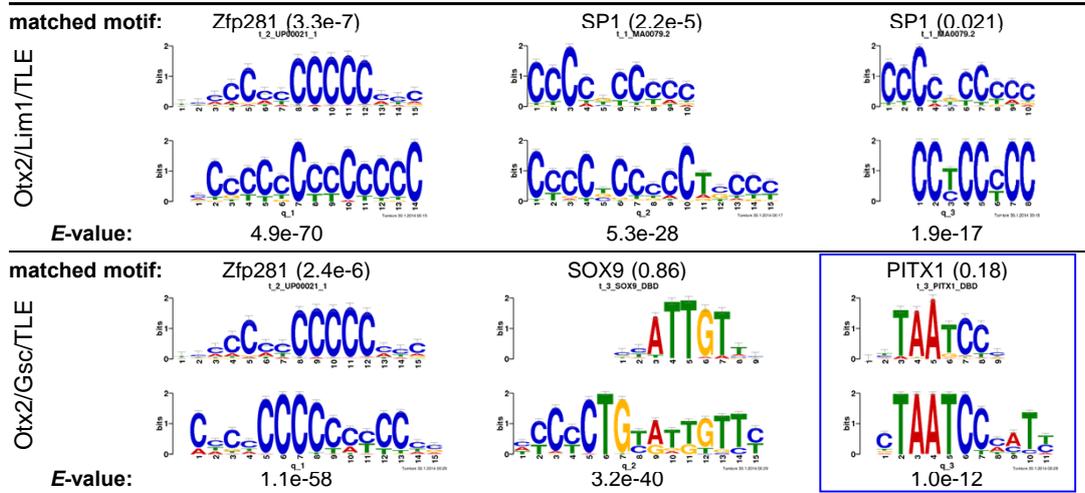
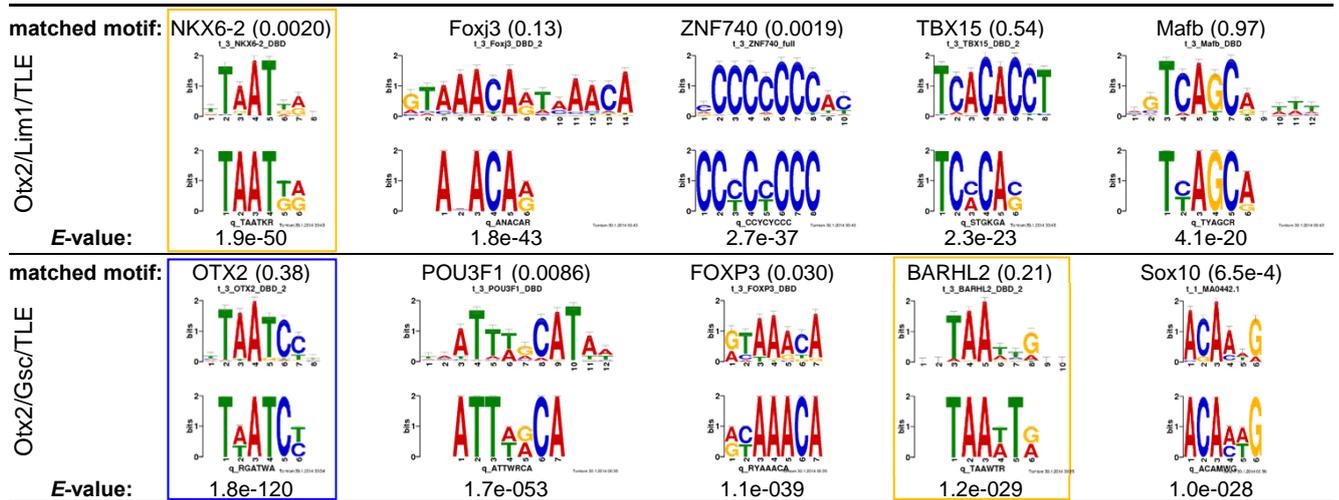


Figure S10 (Yasuoka et al.)

A Motifs discovered by MEME



B Motifs discovered by DREME



C Motifs discovered by Weeder

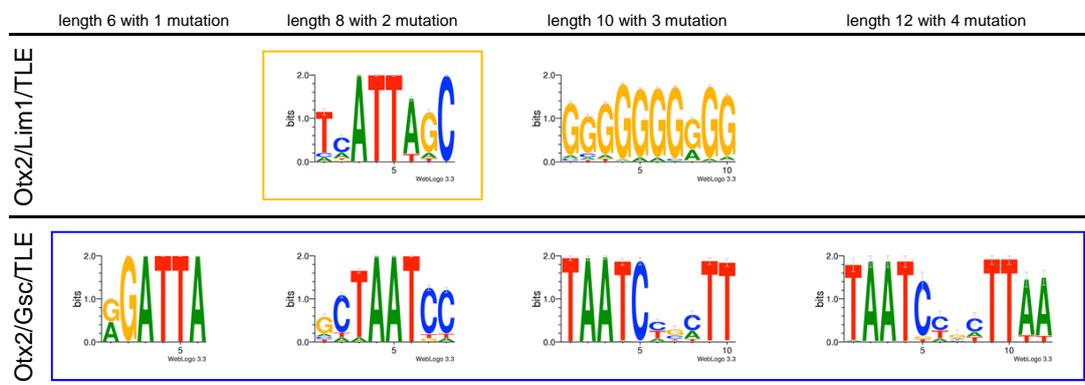


Figure S11 (Yasuoka et al.)

